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# Research toward Control of Key Pecan Insect Pests Using Biorational Pesticides

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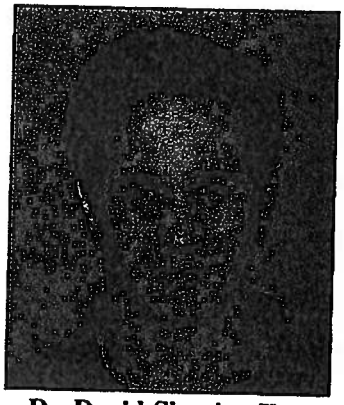
**Summary**

Key pecan insect pests include the black pecan aphid, *Melanocallis caryaefoliae*, pecan weevil, *Curculio caryae*, and stink bugs (Hemiptera: Pentatomidae). Alternative control measures are needed for management of these pests in organic and conventional systems. Our objective was to investigate the potential utility of several alternative insecticides including three plant extract materials (two citrus extract formulations and a eucalyptus extract), and two microbial insecticides, a bacterium, *Chromobacterium subtsugae* (Grandevo™) and a fungus, *Isaria fumosorosea*. In the laboratory, eucalyptus extract, citrus extract, and the bacterium (*C. subtsugae*) caused black pecan aphid mortality. In field tests, combined applications of the fungus (*I. fumosorosea*) plus eucalyptus extract were synergistic and caused up to 82%



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mortality in black pecan aphid. Also in field tests, the bacterium (*C. subtsugae*) reduced adult pecan weevil damage by 55% within the first 3 days post-application, and caused 74.5% corrected mortality within 7 days post-application. Applications of the bacterium (*C. subtsugae*) for suppression of pecan weevil, and eucalyptus extract plus the fungus, *I. fumosorosea*, for control of black pecan aphid show promise as alternative insecticides and should be evaluated further (Shapiro-Ilan et al. 2013).

**Introduction**

Without proper management, several arthropod pests and plant pathogens can cause devastating crop losses in pecan. Insect pests in the Southeastern US of primary economic concern include the pecan weevil and the pecan aphid complex, especially the black pecan aphid. Additionally, stink bugs such as the brown stink bug can also cause significant crop loss (Hudson 2007).

Current control recommendations for key pecan pests include the use of both broad and narrow spectrum insecticides. Although proper use of these insecticides can achieve pest control, some broad spectrum insecticides also kill natural enemies thus flaring populations of some pest species. Overuse of some narrow spectrum of chemistries has led to pest resistance. Therefore, research

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toward the development of alternative approaches for use in conventionally managed orchards is warranted and the lack of options in organically managed orchards has increased the urgency to develop compatible pest management solutions.

Suitable pest management alternatives might be found through the use of biorational insecticides, which are based on naturally occurring organisms or their derivatives. In general, biorational insecticides are expected to be advantageous relative to broad spectrum chemicals in terms of narrow specificity leading to safety toward non-target organisms. Biorational insecticides include botanical insecticides, microbial insecticides, and semiochemicals (e.g., pheromones or other attractants). The objective of this study was to determine the potential of five biorational insecticides to suppress key pecan insect pests. The materials tested included three formulations of plant extracts, i.e., eucalyptus extract, citrus extract at 8.92%, and citrus extract at 19.4%, and two microbial insecticides the bacterium, *Chromobacterium subtsugae* (Grandevo™), and the fungus, *Isaria fumosorosea*.

In laboratory studies, the entomopathogenic fungus, *I. fumosorosea* had high virulence toward pecan aphids (Shapiro-Ilan et al. 2008). Extracts of citrus and eucalyptus have been shown to have insecticidal activity against soft-bodied insects such as scale insects and mealy bugs. The bacterium, *C. subtsugae* was found to be toxic to various insect pests including the Colorado potato beetle, *Leptinotarsa decemlineata*, southern corn rootworm, *Diabrotica undecimpunctata*, Southern green stink bug, Nezara viridula, and sweet potato whitefly, Bemisia tabaci (Martin et al. 2007a, b).

**Materials and Methods**

**Experimental Plan.** A summary of experiments and treatments testing biorational insecticides is presented in Table 1. A total of five experiments were conducted (three laboratory experiments and two field experiments). All laboratory and field experiments were conducted at the USDA-ARS Southeastern Fruit and Tree Nut Research Unit, Byron, GA USA. The results of all experiments were analyzed using standard statistical procedures.

**Experiment 1: Laboratory Experiments Targeting the Brown Stink Bug.** Oral toxicity of the bacterium (*C. subtsugae* at 22.4 g/liter) and eucalyptus extract to brown stink bug was assessed by exposing insects to pre-treated green beans. Five green beans were dipped in the bacterial suspension, eucalyptus extract (at 3% vol/vol), or a water control, and placed on a support (made from hardware cloth) raised about 3 cm off the bottom of a plastic box. After beans had dried, ten adult brown stink bugs were added to each box. The raised support allowed insects to access the treated beans from above or below.

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Insect mortality was determined 7 days post-treatment. There were four replicate boxes for each treatment and control, and the experiment was repeated twice.

**Experiment 2: Laboratory Experiment Targeting Black Pecan Aphid.** Virulence assays were conducted based on procedures described Shapiro-Ilan et al. (2008). Biorational pesticides were applied to aphids using an Automatic Potter Spray Tower. The spray tower was equipped with an intermediate atomizer and delivered 2 ml of treatment at 69 kpa with an allowance of a 5 s settling period. The bacterium (*C. subtsugae*) and eucalyptus extract were applied to 21-24 fourth instar black pecan aphids, which were on 10 leaf discs in a single Petri dish containing water-agar. Two milliliters of suspension was applied to each dish. The bacteria were applied at a rate of 22.4 g/liter, and eucalyptus extract was applied at 3% (vol/vol). There were five replicate dishes of each treatment and the experiment was repeated once in time. In each trial, a water control was applied to an equal number of dishes. The dishes were incubated at 25 oC and aphi mortality was assessed after 2 and 4 d.

**Table 1. Summary of experiments testing suppression of pecan pests**

| Experiment (arena) | Insect target/stage | Treatments | Results |
|---|---|---|---|
| 1 (Lab) | Brown stink bug | Bacteria (*C. subtsugae*), eucalyptus | In text only |
| 2 (Lab) | Black pecan aphid | Bacteria (*C. subtsugae*), eucalyptus | Figure 1 |
| 3 (Lab) | Black pecan aphid | Citrus extract | Figure 2 |
| 4 (Field) | Black pecan aphid | eucalyptus, fungus (*I. fumosorosea*) | Figure 3 |
| 5 (Field) | Pecan weevil | Bacteria (*C. subtsugae*) | Figure 4 |

**Table 1. Summary of experiments testing suppression of pecan pests**

**Experiment 3: Citrus Extract vs. Black Pecan Aphid in the Laboratory.** The two citrus extract formulations (8.92% and 19.3%) were applied to adult black

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pecan aphids following procedures described in Experiment 2. Both materials were applied at 2% (vol/vol). There were 10 aphids per plate, mortality was assessed daily up to 5 d post-treatment, and the experiment was repeated once.

**Experiment 4: Field Suppression of Black Pecan Aphid with Eucalyptus Extract and *I. fumosorosea*.** A field experiment to determine the potential of eucalyptus extract and the fungus, *I. fumosorosea*, to suppress black pecan aphid was conducted in 2010 and 2011; the combination of the two treatments (eucalyptus extract plus the fungus) was also assessed. Eucalyptus extract was chosen for assessment based on relatively high toxicity observed in the laboratory (see Results section), and the entomopathogenic fungus *I. fumosorosea* was selected based on prior research indicating high virulence in the laboratory (Shapiro-Ilan et al. 2008). Blastospores of the fungus (NRRL 3581) were produced in liquid culture as described by Jackson (Jackson et al. 2003). In 2010, the trial was conducted in an unsprayed block of 'Caddo' cultivar pecans. On each tree, leaves on two terminals were sprayed to runoff with 10% eucalyptus extract (vol/vol), fungus at 8.5 x $10^8$ blastospores per ml, a combination of the two treatments, or a water control. Treatments were applied on August 16, 2010. The intention was to apply all treatments using a 7.6 l handheld pump sprayer. However, the treatments that included fungus (i.e., the fungus treatment alone or in combination with eucalyptus extract) caused the sprayers to clog. Therefore, leaves designated for treatment with fungus or the combination treatment were dipped in their respective suspensions rather than being sprayed (the control and the eucalyptus extract -only treatment were sprayed onto the foliage). After treatment, an organdy sleeve cage was placed over each terminal. Terminals were removed from the trees five days post-treatment and the percentage black pecan aphid mortality was determined. There were three replicate trees for each treatment and control. In 2011, the experiment was repeated in a similar manner except that a liquid formulation of the fungus was used (at 8.3 x $10^8$ per ml). The liquid formulation did not clog the sprayers and therefore all treatments were applied via pump sprayers as described above. A 'Stuart' cultivar block was used in lieu of the Caddo block because the latter had a relatively low population of black pecan aphids. Applications were made on September 22, 2011. All other parameters were the same as those described above for the 2010 trial.

**Experiment 5: Field Suppression of Pecan Weevil with the Bacterium, *C. subtsugae*.** Based on the relatively high level of pecan weevil damage suppression observed in an earlier laboratory test (Shapiro-Ilan et al. 2013) an experiment was conducted in 2011 to determine the ability of *C. subtsugae* to suppress damage and cause pecan weevil mortality under field conditions. The experiment was conducted in a mixed-cultivar orchard in Byron, GA. 'Desirable' cultivar trees were used in the experiment. Pre-existing pecan weevil damage to nuts was circled with a fine permanent marker on 32 nut clusters. *C. subtsugae* (9% vol/vol) with 0.01% Tween was applied randomly to half the nut clusters and water (with 0.01% Tween) was applied to the other half. The applications were made by spraying each cluster to run-off using handheld pump sprayers. Eight treated clusters and eight control clusters were caged using netted sleeves (as describe above) and three pairs (three female and three male) of adult weevils collected from Circle traps were added to each sleeve cage. The remaining eight treated clusters and eight control clusters were left without cages. Weevils in plots that lacked cages could choose to feed or oviposit on treated nuts, control nuts, or other nuts in the orchard, whereas the cages created a no-choice dimension to the test. Three days after treatment, four of the clusters in each combination (four replicates) were removed to the laboratory for assessment. The other half of the clusters was assessed 7 days after treatment. The number of new damage sites was assessed for all clusters, whereas weevil survival was only assessed in caged clusters. The test was repeated in a second trial initiated on August 26, 2011.

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**Results**

**Experiment 1: Laboratory Experiments Targeting the Brown Stink Bug.** Toxicity was not detected after green beans were treated with the bacterium, *C. subtsugae* or eucalyptus extract and fed to brown stink bugs. Percentage stink bug mortality 7 days post-application was 2.5%, 5.0%, and 8.75% in the control, *C. subtsugae*, and eucalyptus extract treatments, respectively.

**Experiment 2: Laboratory Experiment Targeting Black Pecan Aphid.** At 2 days post-application, higher aphid mortality was observed in the eucalyptus extract treatment than the control or bacterium (*C. subtsugae*) treatment (Figure 1). Four days post-application both treatments caused higher mortality in black pecan aphids than the control; mortality in the eucalyptus extract treatment was higher than in the *C. subtsugae* treatment (Figure 1).

**Experiment 3: Citrus Extract vs. Black Pecan Aphid in the Laboratory.** Both treatments caused higher mortality relative to the control on all assessment dates (Figure 2). Citrus extract-19.4% and citrus extract-8.92% caused similar levels of aphid mortality (?80-90%) except 5 d post-application mortality was higher in the citrus extract-19.4% treatment (Figure 2).

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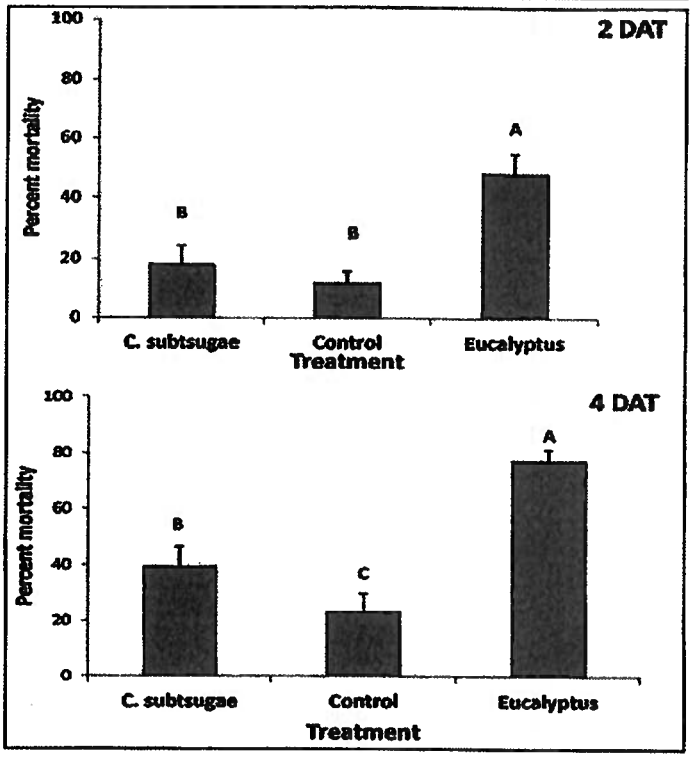


**Figure 1.** Percentage mortality of fourth instar black pecan aphids in the laboratory 2 and 4 days after treatment (DAT) with bacteria (*C. subtsugae*), eucalyptus extract, or a control.

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**Experiment 4: Field Suppression of Black Pecan Aphid with Eucalyptus Extract and *I. fumosorosea*.**

In 2010, aphid mortality was higher in all treatments than the control and no differences were detected among the three treatments (Figure 3). Based on Abbott's formula (correcting for natural mortality, Abbott, 1925) the level of control achieved was 51%, 44%, and 62% in the eucalyptus extract, fungus (*I. fumosorosea*), and combination treatments, respectively. In 2011, only the combination treatment (eucalyptus extract plus fungus) caused higher mortality than the control, whereas mortality in the single-treatment applications was intermediate between the control and combination and not different from either

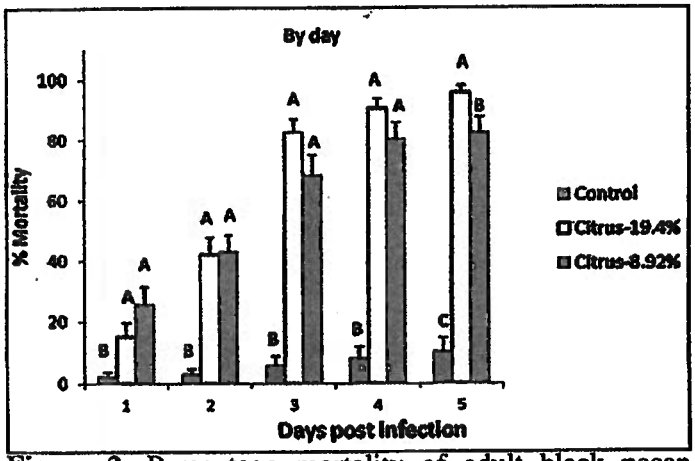


**Figure 2.** Percentage mortality of adult black pecan aphids in the laboratory 1-5 days after treatment (DAT) with citrus extract-19.4%, citrus extract-8.92%, or a control.

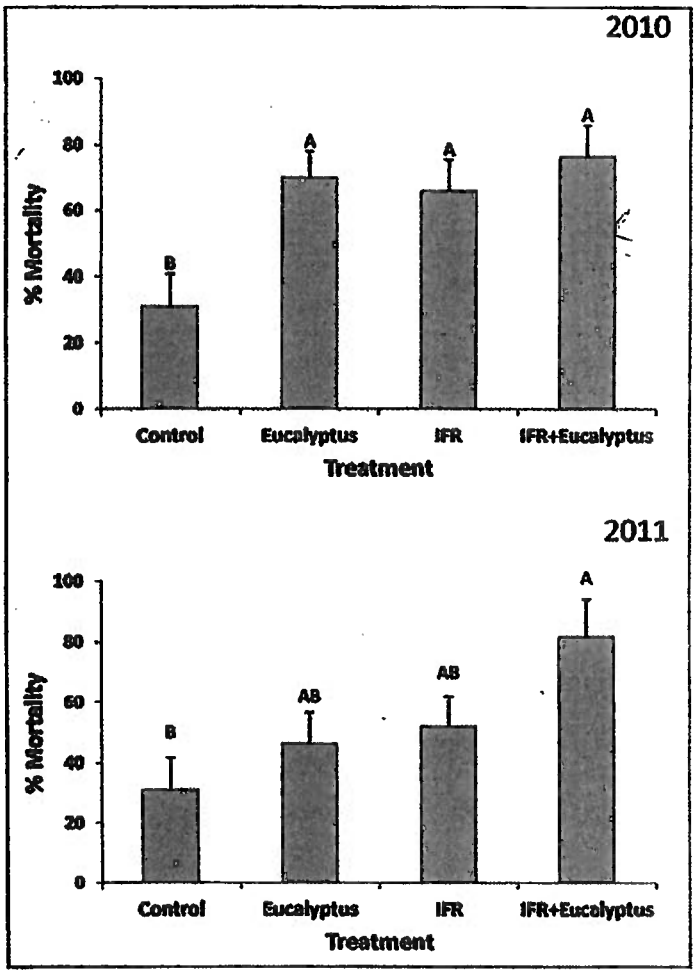


**Figure 3.** Percentage mortality of black pecan aphids following field applications of eucalyptus extract, a fungus, *I. fumosorosea*, (IFR) a combination treatment, or a control.

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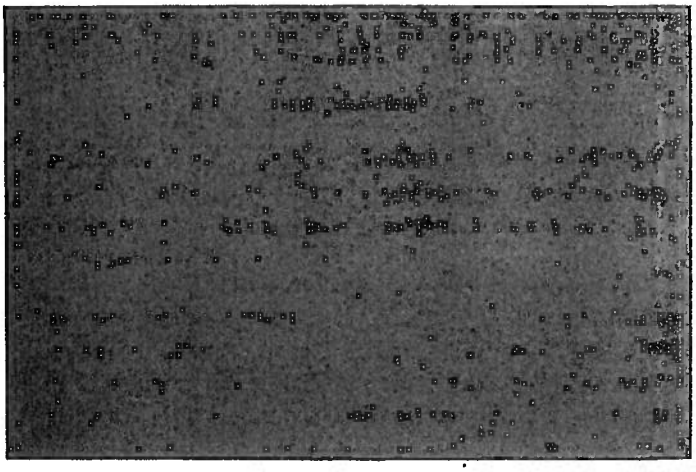

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(Figure 3). The level of control achieved in the combination treatment was 73.5% (based on Abbott's formula). The interaction between eucalyptus extract and *I. fumosorosea* was found to be additive in 2010 and synergistic in 2011.

**Experiment 5: Field Suppression of Pecan Weevil with the Bacterium, *C. subtsugae*.**

In the analysis of nut damage, the interaction between cage effects and treatment effects was not significant; therefore data on number of damage sites was combined across main effects. Three days post-treatment, the number of damage sites on nut clusters treated with C. subtsugae (Grandevo™) was less than the number on the control clusters (Figure 4); based on Abbott's formula the level of damage suppression achieved at 3 days was 56%. In caged nut clusters, adult pecan weevil survival in the bacterial treatment was not different from the control 3 days post-treatment, but weevil survival was lower in treated than non-treated nut clusters 7 days posttreatment (Figure 4); based on Abbott's formula the level of control achieved at 7 days was 75%.

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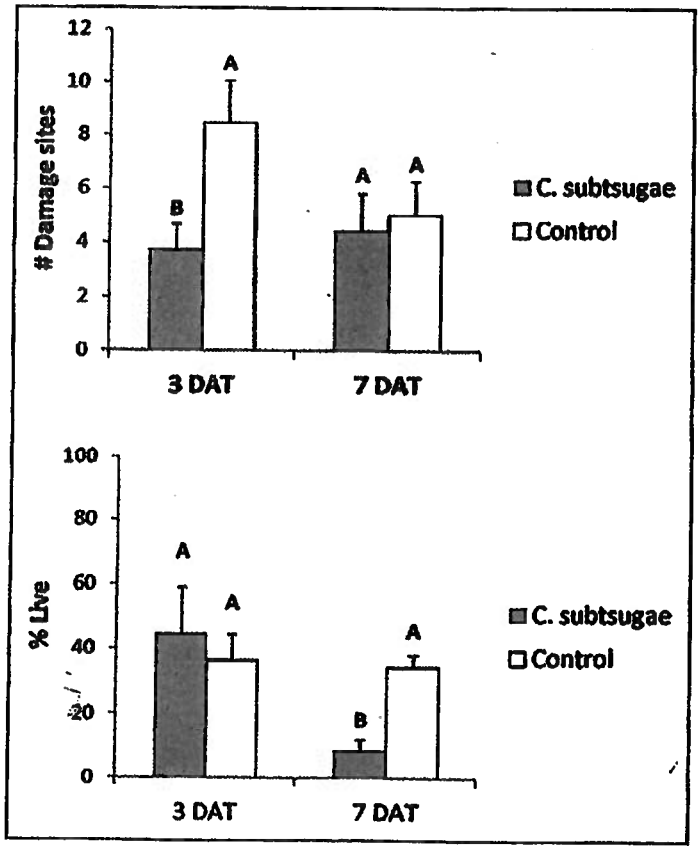


**Figure 4.** Percentage insect survival and number of damage sites on pecan nut clusters due to adult pecan weevil feeding or oviposition following field applications of the bacterium *C. subtsugae* or a control.

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**Discussion and Conclusion**

Our results indicated varying levels of toxicity and efficacy among various biorational insecticides in suppressing key pecan pests. Field applications of *C. subtsugae* (Grandevo™) caused damage reduction and significant mortality in pecan weevil. C. subtsugae has recently been labeled for pecan (as Grandevo™) for control of various pests including pecan weevil, aphids, and pecan nut casebearer, *Acrobasis nuxvorella*. Given that Grandevo™ is OMRI certified, its use could be particularly attractive to organic growers because currently there are no effective organic materials to suppress the pecan weevil once it is in the canopy (though microbial biocontrol agents have been shown to cause high mortality in the soil) (Lacey and Shapiro-Ilan 2008, Shapiro-Ilan and Gardner 2012). Based on our laboratory assays indicating that *C. subtsugae* can cause mortality in black pecan aphids, canopy applications may contribute to simultaneous suppression of at least two key pests.

Despite the high laboratory-based toxicity of eucalyptus extract to black pecan aphids observed in this study, and prior observations of high laboratory virulence in the fungus *I. fumosorosea* (Shapiro-Ilan et al. 2008), these biorational agents only caused significant black pecan aphid suppression in one of the two field trials. Based on our results, it is unlikely either material would be suitable as a stand-alone product for black pecan aphid control unless substantial improvements were made, e.g., in formulation or application methods. In contrast, the combination treatment (fungus plus eucalyptus extract) decreased black pecan aphid survival in both trials. Based on these results, the combination treatment may have potential for commercial suppression of black pecan aphids.

The two formulations of citrus extract (19.4% and 8.92%) produced high levels of toxicity against black pecan aphids in laboratory assays (with citrus extract-19.4% exhibiting higher toxicity). Additional research is warranted to determine the potential of these materials for aphid suppression under field conditions. In contrast, we did not detect any toxic effects of biorational treatments to brown stink bugs.

In conclusion, we have demonstrated that certain biorational pesticides could be developed further for control of key pecan pests: 1) The bacterium *C. subtsugae* (Grandevo™) as a tactic for pecan weevil control, 2) citrus extract for control of black pecan aphids, and 3) the combination of eucalyptus extract with *I. fumosorosea* for suppression of black pecan aphids. These materials may be useful to both organic and conventional growers. Additional experiments (particularly expanded field testing) and cost analysis will be required to determine the

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feasibility of incorporating these biorational agents into an integrated pest management program.

**Acknowledgments**

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This article reports the results of research only. Mention of a proprietary product name does not imply USDA's approval of the product to the exclusion of others that may be suitable.

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